IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	Confirmation No. 2593
Boey *et al.*	Examiner: Gollamudi S. Kishore
Application No.: 09/744,103	Technology Center/Art Unit: 1615
Filed: December 10, 2001	DECLARATION UNDER 37 C.F.R. § 1.132
For: LIPOSOMAL ENCAPSULATED NUCLEIC ACID-COMPLEXES	
Customer No.: 20350	

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Ian MacLachlan, Ph.D., being duly warned that willful false statements and the like are punishable by fine or imprisonment or both, under 18 U.S.C. § 1001, and may jeopardize the validity of the patent application or any patent issuing thereon, state and declare as follows:

1. All statements herein made of my own knowledge are true, and statements made on information or belief are believed to be true and correct.

2. I hold a Ph.D. (1994) from the University of Alberta, and a Bachelor of Science degree (1988) from the University of Alberta. I am presently the Chief Scientific Officer for Protiva Biotherapeutics, Inc. (Burnaby, Canada).

3. My field of expertise is nucleic acid delivery and therapy, including the delivery of siRNA and therapeutic uses thereof. I have authored over thirty-five publications in the field of nucleic acid delivery technology, molecular gene therapy, and molecular genetics. I am a member of the American Society of Gene Therapy and the Oligonucleotide Therapeutics Society.

4. Attached hereto as Exhibit A is a true copy of my *Curriculum Vitae*.

5. I have reviewed and analyzed the above-referenced patent application, and I am familiar with the contents therein. I have also reviewed the contents of the Office Action dated April 18, 2007. It is my understanding that the Examiner is concerned that claims 1-8, 12-13, 15-17, 21-22, 32-39, 43-45, 49, 55, 57, and 59 are anticipated under 35 U.S.C. § 102(a) over U.S. Patent No. 5,908,777 ("Lee *et al.*"); that claims 1-6, 8, 12-13, 15-17, 21-22, 28, 32-37, 39, 43-45, 49, 55, 57, and 59 are anticipated under 35 U.S.C. § 102(a) over U.S. Patent No. 5,891,468 ("Martin *et al.*"); that claims 1-8, 12-13, 15-17, 21-22, 32-39, 43-45, 49, 55, 57, and 59 are anticipated under 35 U.S.C. § 102(a) over Lee *et al.*, *J. Biol. Chem.* 271(14):8481-8487 (1996) ("Lee *et al.* 2 "); that claims 11-14, 26-28, 30-31, 42, 52-53, 56, 58, and 62-63 are obvious under 35 U.S.C. § 103(a) over Lee *et al.* and Lee *et al.* 2; that claims 17-22, 28-29, 45-48, 53-54, 60, and 63-64 are obvious under 35 U.S.C. § 103(a) over Lee *et al.* or Lee *et al.* 2 or Martin *et al.* further in view of U.S. Patent No. 5,885,613 ("Holland"); that claims 8-10, 23-25, 39-40, 50-51, and 61 are obvious under 35 U.S.C. § 103(a) over Lee *et al.* or Lee *et al.* 2 or Martin *et al.* further in view of U.S. Patent No. 6,420,176 ("Lisziewicz"); and that claims 65-66 are obvious under 35 U.S.C. § 103(a) over Lee *et al.* or Lee *et al.* 2 or Martin *et al.* in combination with WO 98/20857 ("Papahadjopoulos"). For the reasons set forth herein, the Examiner' s concerns are overcome.

6. This declaration is provided to clarify the distinguishing elements of the presently claimed invention and that the cited references, *i.e.*, Lee *et al.* , Martin *et al.*, and Lee *et al.* 2, neither teach nor suggest all of the elements of the presently claimed liposomes and, accordingly, do not anticipate the presently claimed invention. This declaration is further provided to demonstrate that the secondary references, *i.e.*, Holland, Lisziewicz, and Papahadjopoulos do not remedy the defects in the cited references and, accordingly, do not render the presently claimed invention obvious.

7. The present invention is directed to liposomes comprising a lipid and a condensing agent-nucleic acid complex ***encapsulated*** within the liposome.

8. It is my understanding that Lee *et al.*, Martin *et al.*, and Lee *et al.* 2 are each cited by the Examiner as allegedly disclosing condensed nucleic acid preparations encapsulated within a liposome and thus, as allegedly anticipating the presently claimed invention. I have reviewed Lee *et al.*, Martin *et al.*, and Lee *et al.* 2, and, as discussed in detail below, ***none*** of Lee *et al.*, Martin *et al.*, or Lee *et al.* 2, teaches or even suggests a liposome comprising a condensing agent-nucleic acid complex ***encapsulated*** within the liposome. It is also my understanding that Holland, Lisziewicz, and Papahadjopoulos are cited by the Examiner as secondary references that in combination with Lee *et al.*, Martin *et al.*, or Lee *et al.* 2, allegedly render the presently claimed invention obvious. I have also reviewed Holland, Lisziewicz, and Papahadjopoulos and, as discussed in detail below, none of the secondary references supply the teachings absent in Lee *et al.* , Martin *et al.*, or Lee *et al.* 2. Accordingly, the combination of the secondary references with Lee *et al.*, Martin *et al.*, or Lee *et al.* 2, does not render the presently claimed invention obvious.

9. Lee *et al.* discloses nucleic acid-lipid complexes comprising anionic liposomes (*see*, col. 8, lines 7-9) and nucleic acid-polylysine complexes (*see*, col. 8, lines23-24). Only after the liposomes are fully formed are they mixed with nucleic acid-polylysine complexes (*see, e.g.*, col. 8, lines 27-29) in deionized water. Lee *et al.* characterizes the interaction between the fully formed liposomes and nucleic acid-polylysine complexes in the liposomes as encapsulation of the nucleic acid-polylysine complex. However, given that DNA does not readily cross lipid membranes, one of skill in the art would appreciate that mixing of a nucleic acid-polylysine complex with preformed liposomes in an aqueous solution does ***not*** result in entrapment of DNA within the internal space of the liposomes, but would, instead, result in formation of nucleic acid-lipid ***complexes***. Without a step that destabilizes the liposome membrane, the nucleic acid would ***not*** be able to enter the liposome and be encapsulated. This is evidenced in the experiments described in heading 13 of this declaration. In these experiments, condensed nucleic acids are mixed with preformed liposomes according to the method of Lee *et al.* The resulting DNA is almost fully accessible to the Picogreen probe, indicating that is indeed is not being encapsulated through the actions of the Lee *et al.* method. This is in direct contrast

to the results of experiments performed with the method described in the instant specification. Using the method of the current application, 41% of the total DNA is rendered inaccessible to the Picogreen probe and is thus liposomally encapsulated. Therefore, in contrast to the presently claimed liposomes, the nucleic acid-lipid ***complexes*** of Lee *et al.* do not comprise a nucleic acid fully encapsulated in a liposome.

10. It is my understanding that the Examiner has also alleged that Lee *et al.* clearly shows the encapsulation of a DNA/polycation complex in Figure 1. It is my expert scientific opinion that Figure 1 of Lee *et al.* does not properly illustrate the results of the methods taught in the specification. As evidenced by the experiments described under heading 13 of this declaration, the methods of Lee *et al.* do not result in the liposomal encapsulation of DNA. The experiment shows that when the methods of Lee *et al.* are employed, that only 2.5% of the total DNA is inaccessible to the Picogreen probe. It is my expert scientific opinion that this 2.5% DNA is ***not liposomally encapsulated***, but rather exists in a state as shown in the third step of Figure 1 of Lee *et al.* This state consists of an intact liposome wrapping its ***exterior*** around the DNA/Polycation complex, as illustrated with the 'Pacman'-like cartoon. In this state, the DNA is less accessible to the Picogreen than when free in solution, resulting in the reduced signal generated in the experiments described below.

Further, it is my opinion that the resulting cartoon representation of the alleged encapsulated DNA is in fact a representation of the alleged encapsulation of ***non-complexed*** DNA. I respectfully draw the Examiner's attention to the upper right corner of the figure, in which Lee *et al.* represent the polycation condensing agent with '+' signs. In the next step of the process, the polycations are shown surrounding the condensed DNA, as represented by a circle containing a number of dots. The following step then shows the 'Pacman'-like structure and I note that the polycations are still surrounding the condensed DNA. However, in the final panel of the figure, it is pointed out that the poycation condensing agents are no longer surrounding the DNA. Rather, the polycations are now surrounding the exterior of the allegedly resulting

liposome. Thus, the DNA allegedly encapsulated in the final panel is ***no longer complexed*** with the polycation condensing agents.

11. Martin *et al.* also disclose nucleic acid lipid complexes. As explicitly set forth in Example 9, preformed liposomes are ***complexed*** to plasmid-histone complexes (*see*, Example 9 at col. 29, lines 41-42). Thus, Martin *et al.* do not describe nucleic acid-histone complexes fully encapsulated in a liposome. Martin *et al.* also describe the use of dehydration-rehydration-extrusion methods to allegedly encapsulate plasmid-histone complexes (*see*, Example 11 at col. 31, lines 11-26). However, as demonstrated in heading 13 below, such methods do not produce liposomes encapsulating nucleic acids. These experiments show that when the methods of Martin *et al. et al.* are employed, all of the pre-condensed DNA is left accessible to the Picogreen probe, and therefore that none of it is actually being encapsulated. Thus, in contrast to the liposomes of the present invention, the nucleic acid-lipid complexes of Martin *et al.* do not comprise a nucleic acid fully encapsulated in a liposome.

12. Lee *et al.* 2 discloses nucleic acid-lipid complexes that are the same or similar to those set forth in Lee *et al.* As set forth in Lee *et al.* 2, preformed anionic liposomes are mixed with nucleic acid-polylysine complexes in deionized water (*see, e.g.*, page 8482, col. 2, lines 13-15). As discussed above in heading 9 and evidenced by the experiments described in heading 13, one of skill in the art would appreciate that mixing preformed liposomes with nucleic acid-polylysine complexes in an aqueous solution would result in formation of lipoplexes, *i.e.*, complexes between the liposomes and nucleic acid-condensing agent, and not liposomes fully encapsulating a nucleic acid. Thus, in contrast to the presently claimed liposomes, the nucleic acid-lipid complexes of Lee *et al.* 2 also do not comprise a nucleic acid fully encapsulated in a liposome.

13. In order to address the Examiner's concerns regarding the speculative nature of the arguments put forth above, a set of experiments was performed under my supervision. These experiments demonstrate that the nucleic acid-lipid complexes of Lee *et al.*,

and Martin *et al.* ***do not*** comprise nucleic acids fully encapsulated within a liposome. Lipid formulations were prepared by first dissolving lipid components in chloroform, combining and then drying off the chloroform with nitrogen gas to produce a lipid film. The lipid composition contained the neutral fusogenic lipid DOPE, the anionic lipid DOPS, and PEG-Cer-C20 at a molar ratio of 83:7:10. Additionally, 0.05% (by mol) of the fluorescent lipid Rhodamine-DOPE was incorporated into the lipid formulations in order to determine the lipid concentrations in the formulated liposomes. Polycation:plasmid complexes were prepared using 25K branched polyethylenimine (PEI) and plasmid DNA at a weight:weight ratio of 4:1. The pre-condensed plasmid DNA was combined with lipids or preformed liposomes at a ratio of 10 mg lipid: 0.4 mg DNA. Lipid vesicle samples were prepared by either extrusion methods, as in Lee *et al.* and Martin *et al.*, or detergent dialysis, as in the current application. For extrusion, liposomes were prepared by hydrating lipid films in distilled water with or without polyplex, followed by extrusion 10 times through 2 stacked 100 nm polycarbonate filters using a 10-mL Extruder (Northern Lipids Inc.) and nitrogen gas at 200-500 psi. Liposome samples were prepared by dialysis after hydrating lipid films using 200 mM octylglucopyranoside (OGP) with or without polyplex. All lipid vesicles without polyplex were prepared at 20 mg/mL lipid, whereas all vesicles prepared in the presence of polyplex were prepared at 5 mg/mL lipid and 0.2 mg/mL pTK27 DNA, including the complexation of lipid vesicles with polyplex.

Particle size analysis was performed on lipid samples with and without polyplex present using a Malvern Instruments Zetasizer. Nucleic acid encapsulation was determined using the membrane impermeable probe Picogreen that fluoresces in the presence of DNA. The proportion of nucleic acid entrapped with liposomes was determined by measuring the fluorescence intensity of these probes before and after the addition of the detergent Triton X-100 in the presence of dextran sulfate to disperse the PEI-DNA complexes. Determination of DNA encapsulation is only possible after the PEI-DNA polyplexes have been broken up using Dextran sulfate at a 10:1 PEI mass ratio. The end point for the liberation of the DNA can be determined by comparing the total DNA detected in the sample compared to input material.

Using the detergent dialysis approach, as taught in the instant specification, homogenous DOPE:DOPS vesicles could only be prepared when PEG-Cer-C20 was present in the formulation. Without PEG-lipid these vesicles had mean diameter of 203 nm with a very high polydispersity value of 0.93. Furthermore, we were able to show that PEI-DNA polyplex was highly encapsulated, 41% incorporation, in liposomes having an average diameter of less than 100 nm (*see,* Table 1 included as Exhibit B). In stark contrast, attempted encapsulation of polyplex by lipid film hydration and extrusion methods, as taught in Lee *et al.* and Martin *et al.*, was not successful. When liposomes were preformed by the extrusion method and subsequently mixed with polyplex, as in Lee *et al.*, only 2.5% of the polyplex was inaccessible to the Picogreen probe. Similarly, using the methods taught by Martin *et al.* in which the lipid film was hydrated in the presence of polyplex and subsequently extruded to produce liposomes, no polyplex was encapsulated (*see*, Exhibit B). Thus, these experiments clearly show that unlike the instant methods, which result in efficient liposomal encapsulation of polyplex DNA, the methods taught in Lee *et al.* and Martin *et al.* do not result in the liposomal encapsulation of pre-condensed nucleic acid.

14. None of the secondary references, *i.e.*, Holland, Lisziewicz, or Papahadjopoulos remedy the defects in Lee *et al.*, Martin *et al.*, or Lee *et al.* 2. Holland is cited by the Examiner as disclosing the use of PEG-ceramide as a liposome bilayer stabilizing component; Lisziewicz is cited by the Examiner as disclosing the use of PEI as a nucleic acid condensing agent; and Papahadjopoulos is cited by the Examiner as disclosing preparation of liposomes by reverse phase evaporation or detergent dialysis. Each of these allegations is addressed in detail below.

15. Holland discloses the use of PEG-ceramide in a nucleic acid lipid ***complex***. More particularly, Holland *et al.* states:

> Cationic lipids have been used in the transfection of cells in vitro and in vivo. . . .The efficiency of this transfection has often been less than desired, for various reasons. ***One is the tendency for cationic lipids complexed to nucleic acid to form unsatisfactory carriers. These carriers are improved by the inclusion of PEG lipids***.

See, column 12, lines 28-39 of Holland *et al.* (emphasis added).

Clearly, the teachings of Holland are directed to forming nucleic acid-cationic liposome ***complexes***, which are structurally and functionally different from the presently claimed liposomes, wherein the nucleic acid-condensing agent complex is encapsulated in the liposome is resistant in aqueous solution to degradation with a nuclease.

16. Lisziewicz discloses nucleic acid-condensing agent complexes and compares the efficiency and toxicity of PEI and PEI-mannose as a condensing agent (*see, e.g.*, col. 15, lines 45-50). Lisziewicz explicitly states that relative to PEI mannose, PEI (1) is more toxic; (2) requires more DNA to neutralize; and (3) is less efficient for transfection. Thus, based on the disclosure of Lisziewicz, one of skill in the art would not use PEI as a nucleic acid condensing agent. Moreover, in contrast to the presently claimed invention, Lisziewicz does not teach or suggest liposomes comprising a nucleic acid-condensing agent complex encapsulated in the liposome.

17. Papahadjopoulos discloses nucleic acid-lipid complexes formed by mixing preformed liposomes with nucleic acids (*see, e.g.*, page 35, lines 1-21). As explained in detail above, mixing preformed liposomes with nucleic acids will lead to formation of lipoplexes, *i.e.*, complexes between the nucleic acids and liposomes, and will ***not*** lead to encapsulation of the nucleic acid in the liposomes. In fact, the disclosure of Papahadjopoulos explicitly states that the methods described therein are used for forming complexes between preformed liposomes and nucleic acids (*see, e.g.*, page 8, lines 28-29 and page 13, lines 4-17). Papahadjopoulos does not disclose or suggest encapsulating nucleic acids in liposomes using detergent dialysis or reverse phase evaporation.

18. In view of the foregoing, it is my opinion that ***none*** of the cited references, *i.e.*, the Lee *et al.* patent, the Martin *et al.* patent nor the Lee *et al.* publication teach (or even suggest) the presently claimed liposomal formulations because ***none*** of Lee *et al.*, Martin *et al.*, nor Lee *et al.* 2 teach (or even suggest) liposomes, wherein the nucleic acid is encapsulated in the

liposome and is thus resistant in aqueous solution to degradation with a nuclease. In contrast to the teachings of Lee *et al.*, Martin *et al.*, and Lee *et al.* 2, the present invention provides novel liposomes which are produced by forming a complex between a nucleic acid (*e.g.*, oligonucleotides, plasmid DNA, *etc.*) and a condensing agent and the resulting complex is ***encapsulated***, within a liposome. This is in stark contrast to the lipoplexes that would be formed based on the using the methodology set forth in each of the cited references.

It is also my opinion that none of the secondary references, *i.e.*, Holland, Lisziewicz, or Papahadjopoulos remedy the defects of Lee *et al.*, Martin *et al.*, and Lee *et al.* 2 because none of the secondary references teach (or even suggest) liposomes wherein a nucleic acid is encapsulated in the liposome and is therefore resistant in aqueous solution to degradation with a nuclease.

19. The declarant has nothing further to say.

May 20, 2008 // //
Date Ian MacLachlan, Ph.D.

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CURRICULUM VITAE
IAN MACLACHLAN, PH.D.

BIOGRAPHIC DATA

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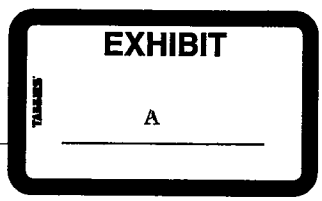
EDUCATION

May 1988 - Jun 1994	**Ph.D. (Biochemistry)** University of Alberta, Edmonton, Canada, & Department of Molecular Genetics, University of Vienna, Austria.
Sep 1985 - May 1988	**B.Sc. (Biochemistry)** University of Alberta, Edmonton, Canada.
Sep 1982 - May 1984	**Biological Sciences** University of Calgary, Calgary, Canada.

EXPERIENCE

Sep 2000 - Present	**Chief Scientific Officer** Protiva Biotherapeutics, Inc., Burnaby, BC, Canada.	Development of Non-Viral Nucleic Acid Delivery Systems for Cancer, Inflammatory, Metabolic and Infectious Disease.
Jul 1996 - Aug 2000	**Team Leader / Research Scientist** Inex Pharmaceuticals Corporation Burnaby, BC, Canada.	Non-Viral Cancer Gene Therapy. Suicide Gene Therapy, Pharmacology,Vector Development, Tumor Modeling, Inducible Gene Expression.
Jul 1994 - Jun 1996	**Research Fellow** Howard Hughes Medical Institute Department of Internal Medicine University of Michigan, USA. Supervisor: Dr. G.J. Nabel	TNF Mediated Activation of NF-κB and the HIV LTR Adenoviral Gene Therapy for Restenosis. The Role of NF-κB in Vertebrate Development.
May 1988 - Jun 1994	**Graduate Student** Lipid and Lipoprotein Research Group University of Alberta, AB, Canada. & Dept. of Molecular Genetics University of Vienna, Austria. Supervisor: Dr. Wolfgang Schneider	Molecular Genetics of the Lipoprotein Receptor Family. Characterization of the Lipoprotein Receptor Mediated Uptake of Riboflavin Binding Protein Including Cloning and Characterization of the *rd* Mutant.
Jan 1988 - Apr 1988	**Undergraduate Research** University of Alberta, AB, Canada. Supervisor: Dr. Wayne Anderson	Computerized Sequence Analysis of Lipoproteins, Crystallography of Membrane Proteins.



Sep 1987 - Dec 1987	**Undergraduate Research** University of Alberta, AB, Canada. Supervisor: Dr. Wolfgang Schneider	Purification and Characterization of Apolipoprotein VLDL-II, an Inhibitor of Lipoprotein Lipase.
Summer 1987	**Undergraduate Research** Bamfield Marine Station, Canada. Supervisor: Dr. Ron Ydenberg	Behavioral Analysis of the Polychaete, *Eudystilia vancouveri.*
May 1983 - Dec 1986	**Computer Programmer** Canadian Hunter Exploration Ltd. Calgary, Alberta, AB, Canada.	Programming of Oil and Gas Reservoir Simulations and Data Analysis Tools Used to Guide the Exploration Efforts of an Oil and Gas Company.

TRAINING

June 2004	American Society of Gene Therapy/ USFDA	Long Term Follow-up of Participants in Human Gene Transfer Research
March 2003	American Society of Gene Therapy / USFDA	Non-Clinical Toxicology in Support of Licensure of Gene Therapies
Sept 2002	Protiva Biotherapeutics	WHMIS and Chemical Safety Retraining
Sept 2002	TLM Consulting	Basic GMP Training
June 2002	American Society of Gene Therapy / USFDA	Clinical Gene Transfer Comprehensive Review Course
Apr 2002	TLM Consulting	Introduction to Gene Therapy Clinical Trials and GLP/GMP
Jul 2001	Protiva Biotherapeutics	Cytotoxic Drug Training
May 2001	American Society of Gene Therapy / USFDA	Clinical Gene Transfer Training Course
Jun – Sep 1998	Leadership Edge Consulting	Lab-to-Leader Training Program Project Management, Coaching, Team Management
Oct 1997	Pape Management Consulting	Project Management Training II
May 1997	University of British Columbia	Radionuclide Safety and Methodology
Feb 1997	Pape Management Consulting	Project Management Training I

AWARDS AND DISTINCTIONS

1995 - 1998	Medical Research Council of Canada Fellowship
1993	Mary Louise Imrie Graduate Award, Faculty of Graduate Studies and Research, University of Alberta
1992 - 1994	Austrian Fonds zur Förderung der Wissenschaftlichen Forschung (Austrian Ministry of Science Scholarship)
1989 - 1993	Heart and Stroke Foundation of Canada Research Trainee
1982	Rutherford Scholarship

AFFILIATIONS AND MEMBERSHIPS

1999 - 2002	Science Council of British Columbia - Health Technology Committee
1998 - Present	American Society of Gene Therapy, Member
2004 - 2007	American Society of Gene Therapy - Non-viral Vectors Committee
2005 - Present	Oligonucleotide Therapeutic Society – Member
2005 - Present	New York Academy of Sciences – Member

SELECTED PATENTS

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Exhibit B

Method to Prepare pre-condensed DNA lipid Particle	No polyplex added		With polyplex		
	Z-avg Size (nm)	Polydispersity	Z-avg Size (nm)	Polydispersity	% DNA Encapsulated
Precondensed DNA lipid particles prepared by Detergent Dialysis approach (Boey Method)	86	0.11	83	0.28	41
Extruded Vesicles, mix with polyplex, with PEG-lipid added after mixing (Martin Method)	120	0.38	171	0.2	0
Extruded Empty Vesicles with PEG-lipid (Lee *et al.* Method)	208	0.24	187	0.21	2.5